Institute of Experimental Morphology, Pathology and Anthropology with Museum
Bulgarian Anatomical Society

Acta Morphologica et Anthropologica, 31 (1-2)
Sofia ● 2024

Neuronal Types in the Rat Spinal Trigeminal Nucleus

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The spinal trigeminal nucleus (SpV) plays a crucial role in the modulation of pain processing. Understanding the morphology of neurons within the subnuclei is essential for unraveling its functional organization. This study aimed to examine the diversity of neuronal morphology in the oral, interpolar, and caudal subnuclei of the SpV. Neurons were characterized based on their soma size and shape using histological staining techniques with hematoxylin and eosin, toluidine blue, and neutral red. We found a broad spectrum of neuronal shapes of at least seven types including boat-shaped, lobulated, round, oval, elongated, pyramidal, triangular, and very large neurons. These findings provide insights into the structural heterogeneity of neurons within the SpV and contribute to our understanding of its complex organization, thus highlighting the significance of neuron shape diversity in sensory processing.

Key words: spinal trigeminal nucleus, neuronal morphology, histological staining, pain processing, rat

Introduction

Nestled within the intricate network of the brainstem lies a structure of paramount importance in sensory processing and pain modulation: the spinal trigeminal nucleus (SpV). This nucleus serves as a critical relay station for somatosensory information originating from the face and head, playing a pivotal role in various sensory and nociceptive pathways [17]. The SpV is anatomically and functionally divided into three distinct subnuclei: the caudalis (SpVc), interpolaris (SpVi), and oralis (SpVo) [13]. Each subnucleus exhibits unique cytoarchitectural features and receives inputs from specific regions of the face and head, thereby contributing to specialized sensory processing. Despite its relatively small size, the SpV plays a multifaceted role in sensory integration, nociception, and autonomic regulation. Sensory information from various modalities, including tactile, thermal, and nociceptive stimuli, converges upon the SpV, where it undergoes intricate processing and modulation [18].

Understanding the structural features, primarily the size and morphology of neurons in these subnuclei, is essential for unraveling the functional organization of the trigeminal sensory system. Prior research has investigated the neuronal architecture of the SpV, providing valuable information on the morphological heterogeneity within its subnuclei [18]. In particular, studies using various histological techniques and immunohistochemistry have repeatedly reported the morphological heterogeneity of neurons within the three subnuclei of the SpV in cats, rats, and humans [5, 6, 7, 9, 16]. The differences observed in these studies highlight the structural complexity of this brain nucleus in various animal species.

Despite the wealth of research on the neuronal size in the SpV, there remains a dearth of studies exploring neuronal shapes comprehensively. This study aims to fill this gap by examining the different shapes of neurons across the three subnuclei in the rat SpV. Utilizing staining techniques, we analyze the morphological heterogeneity of SpV neurons, shedding light on their structural diversity and potential functional implications.

Materials and Methods

The experiments were carried out on adult Wistar rats. A total of 12 male rats with a body weight of 180−300 g were used in our study. All experiments were conducted according to the regulations for work with experimental animals in Bulgaria in compliance with the rules of the Ethics Committee of the Institute of Neurobiology, BAS (registration FWA 00003059 US Department of Health and Human Services) and those of the Research Ethics Committee at the Medical University of Sofia following the Directive 2010/63/EU on the protection of animals used for scientific purposes.

Experimental animals were initially anesthetized with ether followed by an intraperitoneal injection of thiopental (Sigma-Aldrich) at 40 mg/kg dosage. Anesthesia was maintained as the ascending aorta was cannulated via the left ventricle for perfusion. The circulatory system was flushed with 0.05 M phosphate-buffered saline (PBS), pH 7.36, followed by fixation using 4% paraformaldehyde (Merck) in 0.1 M phosphate buffer (PB) for approximately 20 minutes. After brain removal, the region of interest spanning from the midbrain to the upper spinal cord was dissected. Tissue blocks were fixed overnight at 4°C in the same fixative, then washed thoroughly with tap water the following day and finally processed for embedding in paraffin. Tissue sections 7 μm thick were mounted on chrome-gelatinized slides and after rehydration were stained with toluidine blue (500 mg of dye to 100 ml of distilled water) for 5-10 min. This staining was used to describe the cytoarchitectonics of the nucleus and its parts. After dehydration sections were embedded into Entellan (Merck).

The specimens were viewed and photographed using an Olympus VS120-L100 Virtual Slide System research light microscope. the resulting digital images were then saved in TIF format, after which morphometric analysis of the digital images was conducted.

Results

Types of neurons in the spinal trigeminal nucleus

Brainstem sections at the level of SpVc, SpVi, and SpVo were stained to examine SpV subnuclear neurons. SpV neurons were categorized by the shape of their perikarya.

The three subdivisions of SpV were found to include similar types of neurons. Therefore, a general description of these neuronal types will be presented. According to the criteria of the study, each subdivision of the SpV had at least seven types of neurons.

Boat-like neurons

We revealed a unique neuronal phenotype in the rat SpV characterized by its boat-like soma (**Fig. 1A**). The neuronal perikarya resembled boats; they had a broad, elongated shape and a smooth surface viewed with light microscopy. Their somata were found to range in size between 20 and 30 μm, with an average diameter of 25 μm ± 3.8 (n = 11), indicating a significant level of variation among this group of neurons.

Elongated neurons

Other unique anatomical features were evident in medium-sized neurons in the SpV of rats, as shown in **Fig. 1B**. Fusiform or elongated cell bodies were present in these neurons and they belonged to the category of medium-sized neurons. The perikaryal diameter in this population of neurons ranged from 14 to 21 μm, with a mean diameter of 15 μm ± 2.1 (n = 24). This indicates a somewhat variable soma size.

Lobulated neurons

We have further observed another unique anatomical feature that appeared in lobulated neurons in the rat SpV, as shown in **Fig. 1C**. The cell bodies of these neurons were lobulated, meaning that they had irregular surface protrusions or depressions with an average diameter of 16 μm ± 1.3 (n = 9), while their soma diameters ranged from 15 to 21 μm.

Neurons with dilations/swellings at the axonal hillock

Our study also identified spinal trigeminal neurons with distinct morphological characteristics as shown in **Fig. 1D**. Specifically, we have seen neurons on whose cell bodies we observed dilated regions that were particularly visible near the branching points of their processes. There was likely significant dilation of the dendritic architecture at these points, as these dilated regions often exhibited widths that were similar or nearly identical to the diameter of the neuronal cell bodies. The perikarya of neurons with dilations differed significantly from each other in size and shape. This heterogeneity represented a distinctive morphological feature of this type of neuron within the SpV.

Pyramidal neurons

Other spinal trigeminal neurons in the rat SpV with unique morphological features were neurons that have a triangular soma (**Fig. 1E**). We found that this population of neurons exhibited apparent diversity in the mean diameter 17 µm ± 1.2 (n = 12) and a range of soma size from 13 to 21 µm. Dendritic morphology was determined by the presence of two basal and one apical processes in each neuron present in this staining type.

Neurons with an oval cell body and different sizes

We have also identified neurons with various oval or spherical cell bodies with different soma diameters. Their bodies varied from 6 to 20 µm with a mean diameter of 12 µm ± 1.1 (n = 509). This neuronal subtype appeared to have a very heterogeneous soma size (**Fig. 1F**).

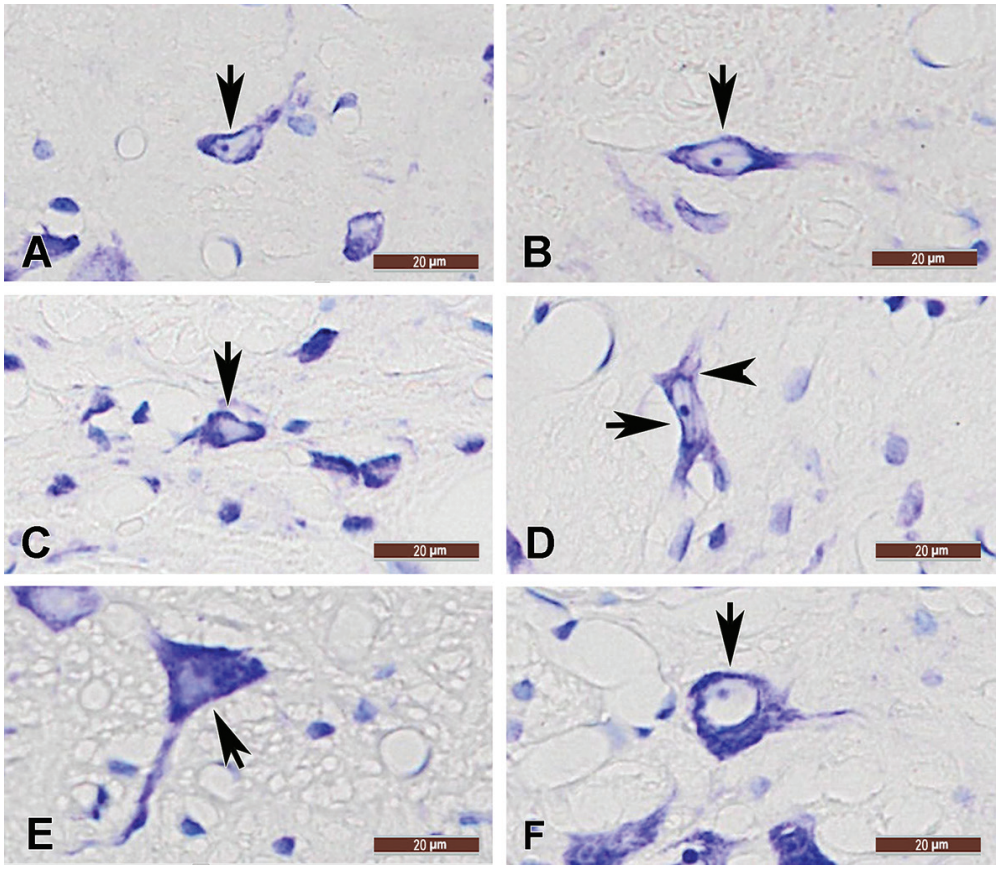

Fig. 1. Morphological types of neurons in the spinal trigeminal nucleus stained with toluidine blue. (**A**) Neuron with a boat-shaped body (arrow); (**B**) Neuron with a bipolar profile and elongated cell body (arrow); (**C**) Neuron with a lobulated cell body (arrow); (**D**) Neuron (arrow) with a dilatation (arrowhead) at the beginning of one of its processes; (**F**) Neuron with a characteristic pyramidal shape of its perikaryon; (**F**) Typical representative of neurons with an oval-shaped cell body (arrow). Scale bar = 20 µm.

Large neurons with different cell body shapes

Another cell population that we observed in the SpV of rats was very large neurons (**Fig. 2**). These neurons possessed large perikarya measuring over 21 µm in soma diameter. Within this population of neurons, the mean soma diameter was 26.7 µm ± 5.5 (n = 33), suggesting that soma size was quite variable. The largest cell body of a neuron that was measured had a diameter of almost 44 µm (43.92 µm). The diversity in somatic morphology among these neurons was reflected in the shape of their somata, which include round, oval, and pear-shaped cells.

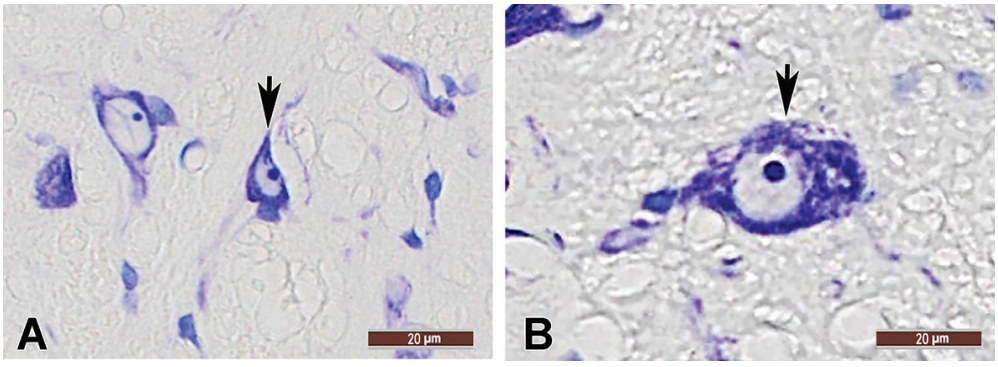

Fig. 2. Neurons with large perikaryal size. Notable diversity in somatic morphology with different shapes of cell bodies arrows described as pear-shaped (A) and oval shapes (B). Scale bar = 20 *µm*.

Discussion

This study represents the first comprehensive investigation of the morphology of neurons in the rat SpV. The results confirm the complex nature of neurons and provide an in-depth description of other neuron types, including fusiform, pyramidal, and multipolar cells, which have only been briefly described in previous reports. The study introduces new findings by describing neurons exhibiting characteristic features such as octopod-like, boat-like, and lobulated cells, and identifies and categorizes at least seven distinct neuronal cell types within the rat SpV, highlighting their similarity in its three parts. In addition, the study revealed pyramidal neurons resembling analogs in other species as well as small round or oval neurons likely correspond to small neurons reported in other species [5, 9, 11]. We also confirmed the similarity of the multipolar neurons in the rat SpV to those reported in previous studies [7, 11].

Prior studies of the morphology of neurons within the SpV, particularly in species such as rats [3, 4, 5, 9, 10], cats [7, 11], camels [2], and humans [15, 16], have primarily categorized these cells based on the size and shape of their bodies using a variety of techniques. In particular, the neuronal elements in the SpV have been identified in different species including rats, cats, monkeys and humans using various examination techniques such as Golgi impregnation, horseradish peroxidase (HRP), Nissl staining and immunocytochemistry, and multiple cell types have been described within the three subdivisions of the SpV [2, 3, 4, 5, 6, 7, 9, 11, 15, 16]. For instance, six distinct

neuron types have been identified in the feline SpV, including pyramidal neurons with spines, pyramidal neurons without spines, multipolar neurons characterized by a dense dendritic tree, and multipolar neurons with sparsely branching dendrites, cells characterized by small oval or round cell bodies and discrete clusters of spines on distal dendrites, and stalked cells showing multiple fine stalked branching and spines on their dendrites [5, 6, 7].

Studies on the human oral subnucleus have delineated two major categories of neurons [15, 16]. The first category consists of small rounded or fusiform cells (8-10 μm in diameter) clustered in small clusters or scattered. The second category comprises large neurons (22 μm in diameter) that have a pear-shaped, fusiform, multipolar, or bipolar morphology.

In addition, two of the subnuclei of the SpV have undergone extensive study in different species. In the feline interpolar subnucleus, for example, observation has revealed the presence of five distinct neuronal cell types [11]. These types exhibit different morphologies, including smooth pyramidal, smooth multipolar characterized by spherical dendritic arborizations, bipolar fusiform or oval with small nuclei, stalked neurons characterized by 2-4 extensively branched spiny dendrites, and cells with very small oval bodies and densely arranged dendrites. The diameters of these neurons range from 6-12 μm to 15-25 μm.

Conversely, three main types of nerve cells have been found in the rat oral subnucleus [3, 4], with reported forms including oval, fusiform, or pyramidal shapes. Cell body sizes of these neurons range from 5-15 μm to 25-50 μm in diameter. Previous studies of spinal trigeminal neurons have categorized them primarily based on the size of their cell bodies paying limited attention to the shape of their perikarya. Notable exceptions include detailed descriptions of stalked neurons in small animals such as rats and cats [5, 9, 11], where neurons were characterized as medium-sized neurons with many spiny stalk numbers. Although two previous studies have briefly outlined dendritic trees of nerve cells in the human oral subnucleus of the spinal trigeminal nucleus [15, 16], it should be noted that pain perception is generally associated with specialized sensory neurons that feature complex dendritic processes for detecting noxious stimuli [8, 12].

Previous studies, particularly those focused on stalk neurons, have provided detailed descriptions characterizing them as complex cells with spiny dendrites and a significant number of spiny dendritic stems [5, 6, 11]. It is noteworthy that the dynamics of spines influenced by neuronal activity and developmental age have been emphasized [14]. In contrast, other neuron types, including islet, fusiform, pyramidal, and multipolar cells, have been mentioned in earlier studies with limited details. In a study on cats, for example, pyramidal neurons were identified, and other neurons were described as small, round or oval with some spines on the dendrites [5]. However, detailed morphological data, such as the density of the dendritic tree and the types and distribution of the various appendages, have not been reported.

An interesting and similar study focused on the morphological characteristics of neurons in the SpV in camels, using the Golgi impregnation method [2]. Additionally, to look at the shape of the neuronal perikarya, the study also classified the cells based on cell body size and shape, dendritic tree density, and the morphology and distribution of appendages. The study identified at least 12 morphological types of neurons, including stalked, islets, octopus-like, lobulated, boat-like, pyramidal, multipolar, round, oval,

and elongated neurons [2]. These neurons exhibited diverse forms of appendages originating from both dendrites and cell bodies, with some featuring large dilatations at dendritic branching points. Taken together with our results, there are two pieces of research done by our awareness, that have successfully been able to show unique cell body morphology such as boat-like and lobulated neurons, one in rats and the other in camels.

Some previous reports emphasized the importance of small and stalked neurons to the exclusion of other types of neurons in the SpV [1, 5]. The present study, however, identified other neuronal types in significant numbers. In particular, the study reported large neurons with a body diameter of up to 44 μm in some cases, a characteristic not documented in the SpV of the rat. Furthermore, neurons with characteristic shapes, such as boat-like, and lobulated cells, were reported exclusively in the rat SpV from this study, with no precedent for similar neurons in this nucleus in rats in other work on this topic.

Conclusion

The neuronal morphologies outlined in this research serve as the foundation for our upcoming inquiries, which aim to elucidate the functional characteristics of these neurons, such as their connections, neurotransmitters, and neuropeptides. This endeavor is part of a broader project focused on compiling a comprehensive database of neuronal cell types across various regions of the rat brain. Within the scope of this study, we identified diverse morphological types of neurons within the rat SpV. Our classification of these neurons relied solely on the size and shape of their cell bodies. Notably, we identified three previously undocumented types of neurons in the rat SpV, known as the boat-like, lobulated, and neurons with dilations at the axonal hillock. We hypothesize that these neuron variations have emerged as adaptations within the rat sensory pathway to respond to painful stimuli. Comparative neuroanatomical investigations are pivotal for advancing our comprehension of the organization of the central nervous system.

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